Tissue inhibitor of metalloproteinase-1 mRNA production and protein secretion are induced by interleukin-1 β in 3T3-L1 adipocytes

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Abstract

The adipokine tissue inhibitor of metalloproteinase (TIMP)-1 is upregulated when weight is gained and promotes adipose tissue development. In the present study, the effect of insulin resistance-inducing and proinflammatory interleukin (IL)-1 β on TIMP-1 gene expression and secretion was investigated in 3T3-L1 adipocytes. Interestingly, protein secretion and mRNA production of TIMP-1 were significantly stimulated by IL-1 β . Thus, IL-1 β induced TIMP-1 secretion in a dose-dependent manner with maximal 3.5-fold upregulation seen at 0.67 ng/ml IL-1 β relative to untreated cells. Furthermore, TIMP-1 mRNA synthesis was significantly stimulated by IL-1 β in a dose-dependent fashion with 2.5-fold induction

seen at IL-1 β concentrations as low as 0.02 ng/ml and maximal 8.1-fold upregulation found at 20 ng/ml effector. Induction of TIMP-1 mRNA was also time dependent with maximal 9.6-fold upregulation detectable after 8 h of IL-1 β treatment. Signaling studies suggested that janus kinase 2 is involved in IL-1 β -induced TIMP-1 mRNA expression. Taken together, our results demonstrate that the TIMP-1 expression is selectively upregulated by proinflammatory IL-1 β , supporting a direct association between insulin resistance, inflammation, and adipose tissue development in obesity.

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Introduction

Obesity is characterized by a chronic inflammatory state. Biologically active proteins secreted from adipose tissue, so-called adipokines, are suggested to link obesity with associated disorders including insulin resistance, type II diabetes mellitus (T2DM), and cardiovascular diseases (Fasshauer & Paschke 2003). Recently, interleukin (IL)-1 β has been characterized as a novel fat-secreted adipokine with insulin resistance-inducing and proinflammatory properties besides tumor necrosis factor (TNF)- α and IL-6 (Lagathu *et al.* 2006, Barksby *et al.* 2007, Jager *et al.* 2007). Thus, chronic IL-1 β treatment impaired insulin-induced glucose transporter (Glut)-4 expression and markedly inhibited its translocation to the plasma membrane through downregulation of insulin receptor substrate-1 (Jager *et al.* 2007). Furthermore, long-term IL-1 α stimulation of 3T3-L1 adipocytes induced expression of suppressor of cytokine signaling-1 and -3 (He *et al.* 2006), which are well-established inhibitors of insulin signal transduction (Fasshauer *et al.* 2004). In addition, IL-1 β dramatically decreased the production of the insulin-sensitizing adipokine adiponectin in 3T3-L1 adipocytes and in human primary fat cells (Lagathu *et al.* 2006). Consistent with these findings, Thomas and co-workers have shown that IL-1

receptor-deficient non-obese diabetic mice were partially protected from the development of T2DM (Thomas *et al.* 2004). Furthermore, blockage of IL-1 signaling with anakinra, a recombinant human IL-1 receptor antagonist, improved glycemic control in patients with T2DM most likely through improved secretory function of β -cells (Larsen *et al.* 2007).

Taking these studies into consideration, identification and characterization of downstream targets of IL-1 β in adipocytes have become a focus of present research since novel pharmacological targets for the treatment of obesity and associated diseases including insulin resistance, T2DM, hypertension, and atherosclerosis might be derived from these studies.

Tissue inhibitor of metalloproteinase (TIMP)-1 is an adipocyte-secreted protein upregulated in obesity that promotes adipose tissue development (Maquoi *et al.* 2002, Chavey *et al.* 2003). Our group has recently shown that TIMP-1 is upregulated both by proinflammatory TNF α and IL-6 (Kralisch *et al.* 2005) and the β -adrenergic agonist isoproterenol in murine adipocytes (Kralisch *et al.* 2006). However, a potential regulation of TIMP-1 by proinflammatory IL-1 β has not been elucidated so far. Therefore, we investigated the effect of IL-1 β on TIMP-1 protein secretion and mRNA production in 3T3-L1 adipocytes in the present study.

Materials and Methods

Materials

Reagents for cell culture were purchased from PAA (Pasching, Austria), and oligonucleotides were from MWG-Biotech (Ebersberg, Germany). AG490, dexamethasone, IL-1 β , isobutylmethylxanthine, LY294002, parthenolide, and PD98059 were obtained from Calbiochem (Bad Soden, Germany), and insulin from Roche Molecular Biochemicals.

Culture and differentiation of 3T3-L1 cells

3T3-L1 adipocytes (American Type Culture Collection, Rockville, MD, USA) were differentiated as described previously (Kralisch *et al.* 2005). In brief, confluent preadipocytes were cultured for 3 days in Dulbecco's modified Eagle's medium (DMEM) containing 25 mM glucose (DMEM-H), 10% fetal bovine serum, and antibiotics (culture medium) further supplemented with 1 μ M insulin, 0.5 mM isobutylmethylxanthine, and 0.1 μ M dexamethasone. After 3 additional days in culture medium with 1 μ M insulin and 3–6 days in culture medium, about 95% of the cells had accumulated fat droplets. All stimulations were carried out in DMEM-H without any additions.

Analysis of TIMP-1 protein secretion

Quantification of TIMP-1 protein secretion into 3T3-L1 cell culture supernatants was performed with a commercially available ELISA from RayBiotech Inc. (Norcross, GA, USA) according to the manufacturer's instructions.

Quantification of mRNA synthesis

The mRNA expression was determined by quantitative real-time RT-PCR in a fluorescent temperature cycler (7000 sequence detection system, Applied Biosystems, Darmstadt, Germany) as described previously (Kralisch *et al.* 2005). In brief, RNA was isolated from 3T3-L1 adipocytes using TRIzol (Invitrogen, Life Technologies, Inc.). After 1 μ g RNA was reverse transcribed with standard reagents (Invitrogen, Life Technologies, Inc.), 2 μ l of each RT reaction were amplified in a 26 μ l PCR. After initial denaturation at 95 °C for 10 min, 40 PCR cycles were performed using the following conditions: 95 °C for 15 s, 60 °C for 1 min, and 72 °C for 1 min. The following primers were used: TIMP-1 (accession no. NM_011593) CTATAGTGCTGGCTGTGGGGTGTG (sense) and TTCCGTGGCAGGCAAGCAAAGT (antisense); TIMP-2 (accession no. NM_011594) GGCCTCCCTCCCTTACTCC (sense) and GACTTCATATTCAGCAGCACAT (antisense); adiponectin (accession no. U37222) AAGGACAAGGCCGTTCTCT (sense) and TATGGGTAGTTGCAGTCAGTTGG (antisense); CCAAT/enhancer-binding protein (C/EBP)- α (accession no. NM_007678) GGTGCGCAAGAGCCGAGATAAAG (sense) and GCCCGCAGCGTGTCAGTT

(antisense); Glut-4 (accession no. NM_009204) GTGGCTCTGCTGCTGGAACG (sense) and GCGGGGG-CCCTGGCTGAAGAG (antisense); peroxisome proliferator-activated receptor (PPAR) γ (accession no. NM_011146) CGTGAAGCCCATCGAGGACATC (sense) and TGGAGCAGGGGGTGAAG (antisense); and 36B4 (accession no. NM007475) AAGCGCGTCCTGGCATTGTCT (sense) and CCGCAGGGGGCAGCAGTGGT (antisense). SYBR Green I fluorescence was monitored after each cycle. Levels of the different genes of interest were quantified using the second derivative maximum method of the ABI Prism 7000 software (Applied Biosystems) determining the crossing points of individual samples. TIMP-1, TIMP-2, adiponectin, C/EBP α , Glut4, and PPAR γ mRNAs were expressed relative to 36B4 that has been used as an internal control due to its resistance to hormonal regulation (Laborda 1991). Specific transcripts were confirmed by melting curve profiles (cooling the sample to 68 °C and heating slowly to 95 °C with measurement of fluorescence) at the end of each PCR, and the specificity of the PCR was further verified by subjecting the amplification products to agarose gel electrophoresis.

Statistical analysis

Results are shown as mean $\pm$ S.E.M. Differences between various treatments were analyzed by Mann–Whitney *U* tests with *P* values <0.01 considered highly significant and <0.05 considered significant.

Results

TIMP-1 mRNA expression is decreased during adipocyte differentiation

We tested whether TIMP-1 mRNA synthesis is modulated during adipocyte differentiation. To this end, TIMP-1 mRNA was quantified in confluent 3T3-L1 preadipocytes (d0), as well as in cells after 3 (d3), 6 (d6), and 9 (d9) days of differentiation induction. TIMP-1 mRNA was highly expressed in preadipocytes (d0) and decreased to 80% at day 3, 40% at day 6 (*P*<0.05), and 30% at day 9 (*P*<0.05) of adipocyte differentiation when compared with day 0 (100%) (Fig. 1A). In contrast, the differentiation markers adiponectin (Fig. 1B), C/EBP α (Fig. 1C), Glut4 (Fig. 1D), and PPAR γ (Fig. 1E) were significantly upregulated in 3T3-L1 cells on days 6 (d6) and 9 (d9) of the differentiation course.

IL-1 β stimulates TIMP-1 protein secretion dose dependently

Differentiated 3T3-L1 cells were treated with various concentrations of IL-1 β for 16 h and TIMP-1 secretion into the medium was measured by ELISA. Interestingly, significant 2.7-fold (*P*<0.05) upregulation of TIMP-1 protein secretion was first seen at effector concentrations as low as 0.2 ng/ml (Fig. 2). Maximal 3.5-fold upregulation

Figure 1 TIMP-1 gene expression is decreased during adipocyte differentiation. Total cellular RNA was isolated at days 0 (0d), 3 (3d), 6 (6d), and 9 (9d) after induction of differentiation and quantitative real-time RT-PCR determining (A) TIMP-1, (B) adiponectin, (C) C/EBP α , (D) Glut4, and (E) PPAR γ mRNA levels normalized to 36B4 expression was performed as described in Materials and Methods. Data are shown relative to cells on day 0 (= 100%) and represent the means $\pm$ S.E.M. of four independent experiments. * $P < 0.05$.

from 1849 (basal) to 6520 pg/ml was detectable at an IL-1 β concentration of 0.67 ng/ml ($P < 0.05$; Fig. 2).

IL-1 β upregulates TIMP-1 mRNA expression in a dose-dependent manner

Treatment with IL-1 β for 16-h-stimulated TIMP-1 gene expression in a dose-dependent manner. Thus, a significant 2.5-fold induction of TIMP-1 mRNA synthesis was detectable at IL-1 β concentrations as low as 0.02 ng/ml ($P < 0.01$; Fig. 3). A maximal 8.1-fold increase was found at 20 ng/ml of the cytokine ($P < 0.01$; Fig. 3). Treatment of 3T3-L1 adipocytes with 20 ng/ml IL-1 β for 16 h did not significantly affect adipocyte differentiation as assessed by Oil Red O staining (data not shown).

IL-1 β upregulates TIMP-1 but not TIMP-2 mRNA expression in a time-dependent manner

Treatment with 20 ng/ml IL-1 β increased TIMP-1 mRNA also in a time-dependent manner (Fig. 4A). Thus, significant fourfold ($P < 0.01$) upregulation was first seen after 2 h of treatment, maximal more than ninefold ($P < 0.01$) induction was observed after 8 h, and significant stimulation persisted for up to 24 h ($P < 0.01$) (Fig. 4A). In contrast, TIMP-2 mRNA production was not affected by IL-1 β treatment of differentiated 3T3-L1 cells (Fig. 4B). Similar to the results obtained in differentiated adipocytes, 20 ng/ml IL-1 β significantly induced TIMP-1 mRNA synthesis time dependently in non-differentiated cells with maximal 2.4-fold ($P < 0.05$) stimulation seen after 4 h of treatment (Fig. 4C).

Signaling molecules mediating the effect of IL-1 β on TIMP-1 mRNA expression

We elucidated which signaling molecules might mediate the effect of IL-1 β on TIMP-1 gene expression. To this end,

Figure 2 TIMP-1 protein secretion is upregulated by IL-1 β . Fully differentiated 3T3-L1 cells were serum deprived for 6 h before the indicated concentrations of IL-1 β were added for 16 h. After this period, TIMP-1 protein levels in the supernatants were determined by ELISA as described in Materials and Methods. Results are the means $\pm$ S.E.M. of three independent experiments. * $P < 0.05$ when compared with control cells.

Figure 3 Dose-dependent upregulation of TIMP-1 mRNA by IL-1 β . Differentiated 3T3-L1 cells were serum starved for 6 h before various concentrations of IL-1 β were added for 16 h. Extraction of total RNA and quantitative real-time RT-PCR determining TIMP-1 mRNA levels normalized to 36B4 expression were performed as described in Materials and Methods. Data are shown relative to untreated control (Con) cells (=100%) and represent the means $\pm$ S.E.M. of seven independent experiments. ** $P < 0.01$ comparing untreated control cells with IL-1 β -treated cells.

3T3-L1 adipocytes were pretreated with specific pharmacological inhibitors of janus kinase (Jak) 2 (AG490, 10 μ M), nuclear factor (NF)- κ B (parthenolide, 50 μ M), p44/42 MAP kinase (PD98059, 50 μ M), or phosphatidylinositol (PI) 3-kinase (LY294002, 10 μ M) for 1 h before IL-1 β (20 ng/ml) was added for 16 h. Treatment of 3T3-L1 adipocytes with AG490, parthenolide, PD98059, and LY294002 for 17 h significantly suppressed basal TIMP-1 expression to 34, 45, 61, and 52% of controls ($P < 0.01$) respectively (Fig. 5). Again, TIMP-1 mRNA synthesis was increased more than eightfold after 16 h of IL-1 β treatment ($P < 0.01$; Fig. 5). IL-1 β -induced TIMP-1 mRNA expression was completely blocked by pharmacological inhibition of Jak2 and NF κ B to 69 and 63% of untreated controls respectively ($P < 0.01$; Fig. 5). In addition, inhibition of p44/42 MAP kinase by PD98059 and PI 3-kinase by LY294002 partially reversed IL-1 β -induced TIMP-1 mRNA synthesis by almost 75 and 60% respectively ($P < 0.01$; Fig. 5).

Synergistic effects of TNF α , IL-6, and IL-1 β on TIMP-1 mRNA expression

Finally, we tested whether IL-1 β , TNF α , and IL-6 might influence TIMP-1 mRNA expression in 3T3-L1 adipocytes synergistically. As shown in Fig. 6, 16-h treatment with IL-1 β (20 ng/ml), TNF α (20 ng/ml), and IL-6 (30 ng/ml) induced TIMP-1 mRNA expression ninefold ($P < 0.05$), fivefold ($P < 0.05$), and sevenfold ($P < 0.05$) respectively. Interestingly, TIMP-1 gene expression further increased when 3T3-L1 cells were treated with IL-1 β and TNF α , IL-1 β and IL-6, or TNF α and IL-6 (Fig. 6). Maximal 85-fold ($P < 0.05$) induction of TIMP-1 mRNA was found after treatment of 3T3-L1 adipocytes with the three cytokines combined (Fig. 6).

Figure 4 Time-dependent upregulation of TIMP-1 but not TIMP-2 mRNA by IL-1 β . (A and B) Fully differentiated 3T3-L1 adipocytes and (C) preadipocytes were serum starved overnight before 20 ng/ml IL-1 β were added for the indicated periods of time. Total RNA was extracted and subjected to quantitative real-time RT-PCR to determine (A and C) TIMP-1 and (B) TIMP-2 mRNA production normalized to 36B4 levels as described in Materials and Methods. Data are expressed relative to untreated control cells (=100%). Results are the means $\pm$ S.E.M. of at least three independent experiments. ** $P < 0.01$ and * $P < 0.05$ comparing IL-1 β -treated with non-treated cells.

Discussion

In the present study, we demonstrate for the first time that IL-1 β induces TIMP-1 gene expression and protein secretion in 3T3-L1 adipocytes. In contrast, TIMP-2 mRNA synthesis is not altered by IL-1 β . These results are in accordance with recent findings from our group showing a specific stimulatory

Figure 5 Signaling molecules involved in IL-1 β -induced TIMP-1 mRNA synthesis. After serum starvation, 3T3-L1 adipocytes were cultured in the presence or absence of AG490 (AG, 10 μ M), parthenolide (Part, 50 μ M), PD98059 (PD, 50 μ M), or LY294002 (LY, 10 μ M) for 1 h before IL-1 β (20 ng/ml) was added for 16 h. Total RNA was extracted and subjected to quantitative real-time RT-PCR to determine TIMP-1 normalized to 36B4 as described in Materials and Methods. Data are expressed relative to non-treated control (Con) cells (=100%). Results are the means $\pm$ S.E.M. of five independent experiments. ** P <0.01 comparing non-treated cells with inhibitor- and IL-1 β -pretreated cells, as well as comparing IL-1 β -treated with inhibitor-pretreated adipocytes.

effect of proinflammatory TNF α and IL-6 on TIMP-1 but not TIMP-2 (Kralisch *et al.* 2005). It is interesting to note in this context that no evidence has been presented so far indicating that TIMP-2 like TIMP-1 influences adipose tissue physiology.

Figure 6 IL-1 β , TNF α , and IL-6 induce TIMP-1 mRNA synergistically. After serum starvation, 3T3-L1 adipocytes were cultured in the presence or absence of IL-1 β (20 ng/ml), TNF α (20 ng/ml), and IL-6 (30 ng/ml) for 16 h. Total RNA was extracted and subjected to quantitative real-time RT-PCR to determine TIMP-1 normalized to 36B4 as described in Materials and Methods. Data are expressed relative to non-treated control cells (=100%). Results are the means $\pm$ S.E.M. of four independent experiments. * P <0.05 comparing effector treated with untreated cells.

During the development of obesity, an extensive reorganization of adipose tissue that involves adipogenesis, angiogenesis, and remodeling of the extracellular matrix is found (Crandall *et al.* 1997). Here, TIMP-1 appears as an interesting candidate promoting fat accumulation. Thus, addition of recombinant TIMP-1 promotes adipogenic accumulation of lipid droplets in 3T3-L1 adipocytes when compared with control cells (Alexander *et al.* 2001). Adipocyte differentiation is accelerated in transgenic mice overexpressing TIMP-1 when compared with wild-type animals (Alexander *et al.* 2001). In addition, the development of obesity is attenuated in TIMP-1-deficient mice on high-fat diet (Lijnen *et al.* 2003). Furthermore, upregulated TIMP-1 expression is found in mice on high-fat diet when compared with standard-fat diet (Maquoi *et al.* 2002). Our group has recently demonstrated that TIMP-1 serum levels are an independent predictor of adiposity in humans with highest levels seen in visceral obesity (Kralisch *et al.* 2007). Furthermore, elevated TIMP-1 serum levels are associated with the presence of carotid artery lesions in dyslipidemic subjects and are related to progression of atherosclerosis (Beaudeau *et al.* 2003). These findings suggest that TIMP-1 is not only a passively regulated novel adipocyte-secreted factor but might also actively maintain adipose tissue mass in states of increased IL-1 β serum levels such as obesity, inflammation, and insulin resistance.

Interestingly, IL-1 β is also a positive inducer of TIMP-1 mRNA synthesis in undifferentiated adipocytes. These results support the notion that IL-1 β is a principal positive regulator of TIMP-1 mRNA expression in adipose tissue where both preadipocytes and adipocytes are present.

We have further elucidated in the present study by which signaling molecules IL-1 β upregulates TIMP-1 expression. Binding of IL-1 β to the extracellular domain of the IL-1 receptor leads to activation of IL-1 receptor-associated kinase (Cao *et al.* 1996) which, in turn, stimulates downstream signaling proteins such as NF κ B (Dinarello 1997). In the present study, we show that pharmacological inhibition of NF κ B by parthenolide reverses IL-1 β -induced TIMP-1 mRNA expression suggesting a potential role of this signaling molecule in IL-1 β -regulated TIMP-1 mRNA synthesis. However, it has to be pointed out that under the conditions studied, parthenolide significantly decreases cell viability assessed by trypan blue staining when compared with untreated 3T3-L1 adipocytes (data not shown). Therefore, we cannot exclude the possibility that downregulation of TIMP-1 after parthenolide pretreatment is due to a toxic effect of this inhibitor. In contrast, AG490, PD98059, and LY294002 do not influence cell viability (data not shown). Jak2 (Doi *et al.* 2002), p44/42 MAP kinase (Stylianou & Saklatvala 1998), and PI 3-kinase (Reddy *et al.* 1997) are downstream signaling molecules of IL-1 β . Jak2 is probably involved in IL-1 β -induced TIMP-1 synthesis in fat cells since inhibition of this signaling protein leads to a complete reversal of the effects of IL-1 β . Pharmacological inhibition of p44/42 MAP kinase and PI 3-kinase downregulates basal TIMP-1

synthesis and partially reverses IL-1 β -induced TIMP-1 mRNA expression. These data indicate that both signaling molecules are principal positive mediators of TIMP-1 gene expression in adipocytes.

Interestingly, TIMP-1 gene expression further increases when 3T3-L1 cells are treated with IL-1 β and TNF α , IL-1 β and IL-6, or TNF α and IL-6. Furthermore, maximal induction of TIMP-1 mRNA is found after treatment with the three cytokines combined. These results indicate that the cytokines tested might work as a network with synergistic effects. Since IL-1 β significantly stimulates IL-6 mRNA and protein expression (data not shown) and IL-6 is a potent inducer of TIMP-1 synthesis (Kralisch *et al.* 2005), it is well possible that IL-6 contributes to IL-1 β -induced TIMP-1.

Taken together, we demonstrate for the first time that IL-1 β is a potent stimulator of TIMP-1 protein secretion and mRNA production in 3T3-L1 adipocytes *in vitro*. Furthermore, we present evidence that the positive effect of IL-1 β is mediated via Jak2.

Declaration of Interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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References

- Alexander CM, Selvarajan S, Mudgett J & Werb Z 2001 Stromelysin-1 regulates adipogenesis during mammary gland involution. *Journal of Cell Biology* **152** 693–703.
- Barksby HE, Lea SR, Preshaw PM & Taylor JJ 2007 The expanding family of interleukin-1 cytokines and their role in destructive inflammatory disorders. *Clinical and Experimental Immunology* **149** 217–225.
- Beaudeux JL, Giral P, Bruckert E, Bernard M, Foglietti MJ & Chapman MJ 2003 Serum matrix metalloproteinase-3 and tissue inhibitor of metalloproteinases-1 as potential markers of carotid atherosclerosis in infraclinical hyperlipidemia. *Atherosclerosis* **169** 139–146.
- Cao Z, Henzel WJ & Gao X 1996 IRAK: a kinase associated with the interleukin-1 receptor. *Science* **271** 1128–1131.
- Chavey C, Mari B, Montheuol MN, Bonnafous S, Anglard P, Van Obberghen E & Tartare-Deckert S 2003 Matrix metalloproteinases are differentially expressed in adipose tissue during obesity and modulate adipocyte differentiation. *Journal of Biological Chemistry* **278** 11888–11896.
- Crandall DL, Hausman GJ & Kral JG 1997 A review of the microcirculation of adipose tissue: anatomic, metabolic, and angiogenic perspectives. *Microcirculation* **4** 211–232.
- Dinareello CA 1997 Interleukin-1. *Cytokine and Growth Factor Reviews* **8** 253–265.
- Doi M, Shichiri M, Katsuyama K, Ishimaru S & Hirata Y 2002 Cytokine-activated Jak-2 is involved in inducible nitric oxide synthase expression independent from NF-kappaB activation in vascular smooth muscle cells. *Atherosclerosis* **160** 123–132.
- Fasshauer M & Paschke R 2003 Regulation of adipocytokines and insulin resistance. *Diabetologia* **46** 1594–1603.
- Fasshauer M, Kralisch S, Klier M, Lossner U, Bluher M, Klein J & Paschke R 2004 Insulin resistance-inducing cytokines differentially regulate SOCS mRNA expression via growth factor – and Jak/Stat signaling pathways in 3T3-L1 adipocytes. *Journal of Endocrinology* **181** 129–138.
- He J, Usui I, Ishizuka K, Kanatani Y, Hiratani K, Iwata M, Bukhari A, Haruta T, Sasaoka T & Kobayashi M 2006 Interleukin-1alpha inhibits insulin signaling with phosphorylating insulin receptor substrate-1 on serine residues in 3T3-L1 adipocytes. *Molecular Endocrinology* **20** 114–124.
- Jager J, Gremeaux T, Cormont M, Marchand-Brustel Y & Tanti JF 2007 Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. *Endocrinology* **148** 241–251.
- Kralisch S, Klein J, Lossner U, Bluher M, Paschke R, Stumvoll M & Fasshauer M 2005 Proinflammatory adipocytokines induce TIMP-1 expression in 3T3-L1 adipocytes. *FEBS Letters* **579** 6417–6422.
- Kralisch S, Lossner U, Bluher M, Paschke R, Stumvoll M & Fasshauer M 2006 TIMP-1 expression and secretion are induced by β -adrenergic stimulation in 3T3-L1 adipocytes. *Journal of Endocrinology* **189** 665–670.
- Kralisch S, Bluher M, Tonjes A, Lossner U, Paschke R, Stumvoll M & Fasshauer M 2007 Tissue inhibitor of metalloproteinase-1 predicts adiposity in humans. *European Journal of Endocrinology* **156** 257–261.
- Laborda J 1991 36B4 cDNA used as an estradiol-independent mRNA control is the cDNA for human acidic ribosomal phosphoprotein PO. *Nucleic Acids Research* **19** 3998.
- Lagathu C, Yvan-Charvet L, Bastard JP, Maachi M, Quignard-Boulange A, Capeau J & Caron M 2006 Long-term treatment with interleukin-1beta induces insulin resistance in murine and human adipocytes. *Diabetologia* **49** 2162–2173.
- Larsen CM, Faulenbach M, Vaag A, Volund A, Ehlers JA, Seifert B, Mandrup-Poulsen T & Donath MY 2007 Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *New England Journal of Medicine* **356** 1517–1526.
- Lijnen HR, Demeulemeester D, Van Hoef B, Collen D & Maquoi E 2003 Deficiency of tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) impairs nutritionally induced obesity in mice. *Thrombosis and Haemostasis* **89** 249–255.
- Maquoi E, Munaut C, Colige A, Collen D & Lijnen HR 2002 Modulation of adipose tissue expression of murine matrix metalloproteinases and their tissue inhibitors with obesity. *Diabetes* **51** 1093–1101.
- Reddy SA, Huang JH & Liao WS 1997 Phosphatidylinositol 3-kinase in interleukin 1 signaling. Physical interaction with the interleukin 1 receptor and requirement in NFkappaB and AP-1 activation. *Journal of Biological Chemistry* **272** 29167–29173.
- Stylianou E & Saklatvala J 1998 Interleukin-1. *International Journal of Biochemistry and Cell Biology* **30** 1075–1079.
- Thomas HE, Irawaty W, Darwiche R, Brodnicki TC, Santamaria P, Allison J & Kay TW 2004 IL-1 receptor deficiency slows progression to diabetes in the NOD mouse. *Diabetes* **53** 113–121.

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